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**REVIEW**

# Surveillance for highly pathogenic avian influenza in wild birds in the USA

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## Abstract

As part of the USA's National Strategy for Pandemic Influenza, an Interagency Strategic Plan for the Early Detection of Highly Pathogenic H5N1 Avian Influenza in Wild Migratory Birds was developed and implemented. From 1 April 2006 through 31 March 2009, 261 946 samples from wild birds and 101 457 wild bird fecal samples were collected in the USA; no highly pathogenic avian influenza was detected. The United States Department of Agriculture, and state and tribal cooperators accounted for 213 115 (81%) of the wild bird samples collected; 31, 27, 21 and 21% of the samples were collected from the Atlantic, Pacific, Central and Mississippi flyways, respectively. More than 250 species of wild birds in all 50 states were sampled. The majority of wild birds (86%) were dabbling ducks, geese, swans and shorebirds. The apparent prevalence of low pathogenic avian influenza viruses during biological years 2007 and 2008 was 9.7 and 11.0%, respectively. The apparent prevalence of H5 and H7 subtypes across all species sampled were 0.5 and 0.06%, respectively. The pooled fecal samples ($n$ = 101 539) positive for low pathogenic avian influenza were 4.0, 6.7 and 4.7% for biological years 2006, 2007 and 2008, respectively. The highly pathogenic early detection system for wild birds developed and implemented in the USA represents the largest coordinated wildlife disease surveillance system ever conducted. This effort provided evidence that wild birds in the USA were free of highly pathogenic avian influenza virus (given the expected minimum prevalence of 0.001%) at the 99.9% confidence level during the surveillance period.

**Key words:** disease surveillance, highly pathogenic avian influenza, H5N1, morbidity and mortality, wild bird.

## INTRODUCTION

Wild birds, specifically species in the order Anseriformes (e.g. ducks, geese and swans) and Charadriiformes (e.g. gulls, terns and shorebirds), are considered the natural reservoir of all 144 subtypes of avian influenza viruses (AIVs), which are globally distributed in these species (Webster *et al*. 1992; Stallknecht & Brown 2008). Avian influenza infections in wild birds are typically apathogenic or subclinical in nature (Webster *et al*. 1992; Stallknecht *et al*. 2007; van Gils 2007). Until the recent emergence of the highly pathogenic avian influenza virus (HPAIV) H5N1 subtype in Asia, severe morbidity and mortality from AIV infection in wild birds was uncommon and documented

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on only one occasion (Becker 1966). After movement of HPAIV H5N1 out of Southeast Asia and into Qinghai Province in China, Mongolia, and eventually into Europe and Africa in 2005, considerable international effort focused on controlling HPAIV H5N1 in endemic countries and preventing further spread.

Wild birds, by their very nature, are not subject to disease containment controls as are domestic birds and people. Therefore, the ability to effectively control the spread of the HPAIV H5N1 virus in these species depends on the ability to rapidly detect the pathogen and available resources to mitigate potential spread to domestic birds. As part of the USA's National Strategy for Pandemic Influenza, which included both animal and human pandemic preparedness, an interagency strategic plan to detect an introduction of HPAIV was developed (Homeland Security Council 2005; USDA 2006). The USA recognized that the greatest risk of introduction was from the illegal importation of poultry and poultry products, and through the illegal trade of wild and exotic birds. Consequently, border protection and domestic bird surveillance programs already in place were strengthened to meet the increased risk of the rapidly spreading HPAIV H5N1 subtype.

The risk that wild birds could move the HPAIV H5N1 subtype into the country was also identified; wild birds likely played a role in moving the virus into Qinghai Province in China, Mongolia and Western Europe (Chen *et al*. 2006a,b; Gilbert *et al*. 2006; Olsen *et al*. 2006; Weber *et al*. 2007; Wang *et al*. 2008; Szeleczky *et al*. 2009). Although studies on AIVs in wild birds have been conducted in the USA and Canada (Olsen *et al*. 2006; Stallknecht *et al*. 2007), these are limited in geographic scope and not designed to provide early warning of new virus introductions. To decrease the risk of an undetected entry of H5N1 and other HPAIV into the country, the USA developed an early detection system for wild birds. A working group of wildlife biologists, veterinarians, virologists and public health experts developed The USA Interagency Strategic Plan for An Early Detection System for Highly Pathogenic H5N1 Avian Influenza in Wild Migratory Birds (USA Strategic Plan) that provided guidelines for agencies and programs conducting AIV surveillance in wild birds (USDA 2006). The purpose of this plan was to describe the essential components of a unified national system for the early detection of HPAIV, specifically the H5N1 subtype, in migratory birds. Although the immediate concern was a potential introduction of HPAIV H5N1 into the USA, the system was developed to detect any HPAIV in migratory birds regardless of the source. Additionally, the system increases knowledge regarding low pathogenic avian influenza viruses and the general health of wild birds. This Plan has been used to develop flyway and state-specific implementation plans for HPAIV surveillance by establishing guidelines consisting of standardized protocols for sampling wild birds, handling and shipping samples, diagnostic testing, and communicating results.

The USA Strategic Plan targets all sick and dead wild birds, as well as wild bird species in North America that have the highest risk of being exposed to, or infected with, the HPAIV H5N1 subtype based on known migratory movement patterns. These include birds that migrate directly between Asia or Europe and the USA that might be in contact with species from areas with reported outbreaks, that are known to be reservoirs of AIV, or that occur in high risk areas. However, should HPAIV H5N1 be detected in domestic birds in the USA, sampling of additional wild species would be conducted.

Sampling for HPAIV in wild birds was stratified longitudinally to account for general migratory patterns across the continent. Although intraspecific and interspecific variability in migratory pathways are common (Hochbaum 1955; Welty & Baptista 1988; Brown *et al*. 2001), the traditional waterfowl flyways (i.e. Atlantic, Mississippi, Central and Pacific) were used as a template in evaluating the risk of HPAIV H5N1 introduction through migratory birds on a continental scale (Lincoln 1935; Blohm 2006). The Pacific and Central Flyways were considered the regions through which the introduction of HPAIV H5N1 most likely would occur by wild birds. Many migratory species that nest in subarctic and arctic Siberia, Alaska and Canada follow the Pacific and Central Flyways to wintering areas in North and South America (Winker *et al*. 2007). The overlap at the northern ends of these flyways with Eurasian flyways establishes a pathway for potential disease transmission across continents and for mixing, re-assortment and exchange of genetic material among strains from Eurasia and North America (Jackwood & Stallknecht 2007; Krauss *et al.* 2007; Koehler *et al*. 2008; Lebarbenchon *et al*. 2009).

Although the risk of HPAIV H5N1 introduction through migratory birds was considered higher in the Pacific and Central Flyways, potential introduction of the virus through the Atlantic and Mississippi Flyways was also considered important. Some species such as the northern pintail (*Anas acuta* L., 1758) and tundra swan (*Cygnus columbianus* Ord, 1815) migrate across several flyways during fall and spring (Lincoln 1935; Kear 2005; Boere & Stroud 2006). In addition, geographic overlap of breeding birds in the Atlantic Flyway with birds from the East Atlantic Flyway exists (Olsen *et al*. 2006), although the de-

gree of interspecific and intraspecific overlap is considerably less than occurs in flyways of the Pacific region (Markova *et al*. 1999; Kear 2005).

Finally, although HPAIV H5N1 had not been detected in the western hemisphere, the potential for wild birds to move the virus north if it was introduced into Central and South America was considered. Therefore, the USA Strategic Plan provided a national framework for HPAIV H5N1 surveillance in wild birds, which recommended that regional flyway plans be developed. These flyway plans were further refined into individual state surveillance plans, such that all the potential routes of entry for HPAIV H5N1 through migratory birds could be monitored.

Our objective is to provide an overview of the comprehensive USA Strategic Plan and its implementation. Several authors have criticized the USA HPAIV H5N1 early detection system, suggesting that it focused exclusively on the Asia–Alaska route of entry into the USA (Kilpatrick *et al*. 2006; Peterson *et al*. 2007; Peterson & Williams 2008). These authors imply that little to no wild bird surveillance in other potential pathways of introduction (e.g. entry from the South and transatlantic routes) was being conducted. Here, we provide a comprehensive description of the US interagency early detection system to clarify previous misconceptions and to provide preliminary data on the first 3 years of surveillance.

## METHODS OF SURVEILLANCE

The USA Strategic Plan recommends five strategies for collecting surveillance data on AIVs in wild birds. Agencies and organizations are encouraged to use one or more of these strategies when designing AIV surveys. Each strategy has biological, logistical and economic benefits and constraints; consequently, agencies have based implementation of the strategies on an evaluation of these factors at specific sampling locations and times of year.

### Investigation of morbidity and mortality events

Highly pathogenic avian influenza H5N1 has been shown to cause morbidity and mortality in a wide variety of wild species (USGS 2008), and most detections in wild birds have been through morbidity and mortality events (Olsen *et al*. 2006; Gauthier-Clerc *et al*. 2007). Systematic investigation of these events in wild birds seems to offer the highest and earliest probability of detecting HPAIV H5N1 if it is introduced by wild birds (Kilpatrick *et al*. 2006; USDA 2006).

Benefits gained from conducting disease investigations of wildlife mortality events are not unique to AIV. Many diseases have been identified through the wildlife disease investigation process (Friend & Franson 1999; McLean *et al*. 2002; Merianos 2007). Investigation of morbidity and mortality events also provides management recommendations that can mitigate or reduce additional events in wild birds. Morbidity and mortality sampling in wild birds is important for providing early warning to domestic animals, wildlife and human health officials. Outbreaks near domestic poultry and swine operations should initiate enhanced surveillance activities on farms and measures to minimize contact among wild birds, domestic animals and humans.

The success of this strategy requires early detection and assessment of events, rapid submission of samples to qualified diagnostic laboratories, rapid testing, immediate reporting of diagnostic results and rapid implementation of pre-established response protocols. The US strategy capitalizes on existing morbidity and mortality surveillance programs by state and federal agencies; some of these programs have been in place for decades (e.g. surveillance at migratory waterfowl refuges) and others are relatively new (e.g. West Nile virus monitoring programs). These programs use agency personnel as well as the public to detect and report events to trained wildlife disease investigators. Investigations related to morbidity and mortality events are conducted regardless of the time of year, type of species involved, number of species involved, or the number of samples previously collected in the state. Assessment of these events, and collection and shipment of samples to diagnostic laboratories are usually made within 24 h of identifying the incident. Diagnostic testing and reporting results are completed within an additional 72 h, allowing for rapid implementation of response protocols.

The USA has enhanced its capabilities to respond to morbidity and mortality events by increasing personnel and resources dedicated to detection, investigation and reporting of sick and dead birds. Training courses designed to increase the number of wildlife professionals qualified to investigate morbidity and mortality events were conducted, educational materials were provided to sportsmen, bird watchers and the general public to increase reporting of events, and a national telephone hotline was established to report dead birds.

Although investigation of morbidity and mortality events in wild birds is critical for effective HPAIV H5N1 detection systems, comprehensive surveillance of these events is problematic, even in countries with established programs. Most morbidity and mortality events in wild

birds go undetected because they involve few individuals, occur in areas of low human density, or quickly become unavailable for sampling due to predation, scavenging or rapid autolysis (Bellrose 1981; Humburg *et al*. 1983; Stutzenbacher *et al*. 1986; Baldassarre & Bolen 2006; Klopfleisch *et al*. 2007). Additionally, evidence for the evolution of HPAIV H5N1 strains that are not pathogenic to particular species of wild birds is mounting (Sturm-Ramirez *et al*. 2004; Hulse-Post *et al*. 2005; Kou *et al*. 2005; Chen *et al*. 2006b). Recent experimental research (Keawcharoen *et al*. 2008) demonstrates that mallards (*Anas platyrhynchos* L., 1758) are resistant to developing clinical signs from HPAIV H5N1 infection, whereas another study documents that even highly susceptible species, such as mute swans (*Cygnus olor* Gmelin 1789) can be clinically protected by previous exposure to AIV (Kalthoff *et al*. 2008). Consequently, surveillance systems should also employ active (e.g. apparently healthy bird) as well as passive (e.g. morbidity/mortality event) sampling techniques (Doherr and Audigé 2001; Guberti & Newman 2007; OIE 2008).

## Surveillance in apparently healthy birds

Two strategies for sampling apparently healthy wild birds are recommended in the USA Strategic Plan: hunter-harvest and live-bird sampling. Similar to morbidity and mortality event sampling, each of these strategies has advantages and disadvantages. Successful implementation of these strategies is time and location specific.

### Hunter-harvest sampling

Regulated hunting of wild migratory birds by sportsmen and subsistence harvests by Native Americans occur throughout most of North America. The primary advantage of hunter-harvest sampling is its cost-effectiveness: most of the waterfowl species in North America are classified as game birds, existing infrastructure (e.g. check stations) is in place in most migratory and wintering areas, and sufficient numbers of birds are harvested by hunters, decreasing the amount of time and resources required by biologists and veterinarians to obtain samples.

The main disadvantages of hunter-harvest sampling are that not all species are harvested and hunting seasons only occur at specific times of the year (e.g. September through January). In addition, although sport hunting is widely distributed throughout North America, specific areas receive little to no hunting pressure because of low hunter density or because it is prohibited by regulation (e. g. urban areas, preserves and private property). Finally, reliable collection of site information (e.g. geographic information system coordinates) might not be available.

### Live-bird sampling

Live-bird sampling involves capturing, sampling and releasing wild birds. This strategy is often time and labor intensive, requiring trained personnel, which can result in a significant financial investment. However, if implemented properly, live-bird sampling provides valuable data toward a comprehensive surveillance system.

An important advantage of this strategy is that it can be implemented at specific sites and at any time of the year birds are present. For example, many species of Charadriiformes are not hunted and hunting of game species within urban areas is not possible. Virtually any species of interest can be targeted, but the technique requires trained biologists to operate specific trap types (e.g. mist nets, cannon and rocket nets, and Q-traps) as well as properly handling targeted species to prevent injury and death.

## Sentinel species

Waterfowl, exhibition game fowl and poultry flocks reared on backyard premises have been used as sentinels for active surveillance for avian diseases of interest to the commercial poultry industry and regulatory agencies (McBride *et al*. 1991; Johnson *et al*. 2004). Sentinel ducks have been used effectively to determine the presence of AIV and timing of infection associated with the arrival of wild migratory waterfowl in wetland habitats (Turek *et al*. 1984; Sinnecker *et al*. 1982a,b; Halvorson *et al*. 1983; Halvorson *et al*. 1985; Kelleher *et al*. 1985).

Major advantages of sentinel bird surveillance include the previous success of such systems to effectively detect AIV (Halvorson *et al*. 1983; Halvorson *et al*. 1985) and the applicability in areas in which other methods cannot be used (e.g. urban areas). Disadvantages include the expense of rearing disease-free birds, pen construction and husbandry. Sentinel flocks are also subject to predation and human disturbance.

## Wild bird fecal sampling

Avian influenza viruses are generally transmitted by waterfowl through the intestinal tract and viable virus can be detected in feces (Slemons & Easterday 1977; Webster *et al*. 1978). Analyses of fecal material from waterfowl habitat can provide evidence of AIV circulating in wild bird populations, the specific subtypes present, levels of pathogenicity, and possible risks to poultry and susceptible livestock (Widjaja *et al*. 2004; McLean *et al*. 2007; Franklin *et al*. 2009). Monitoring of fecal samples gathered from waterfowl habitat is a reasonably cost effective

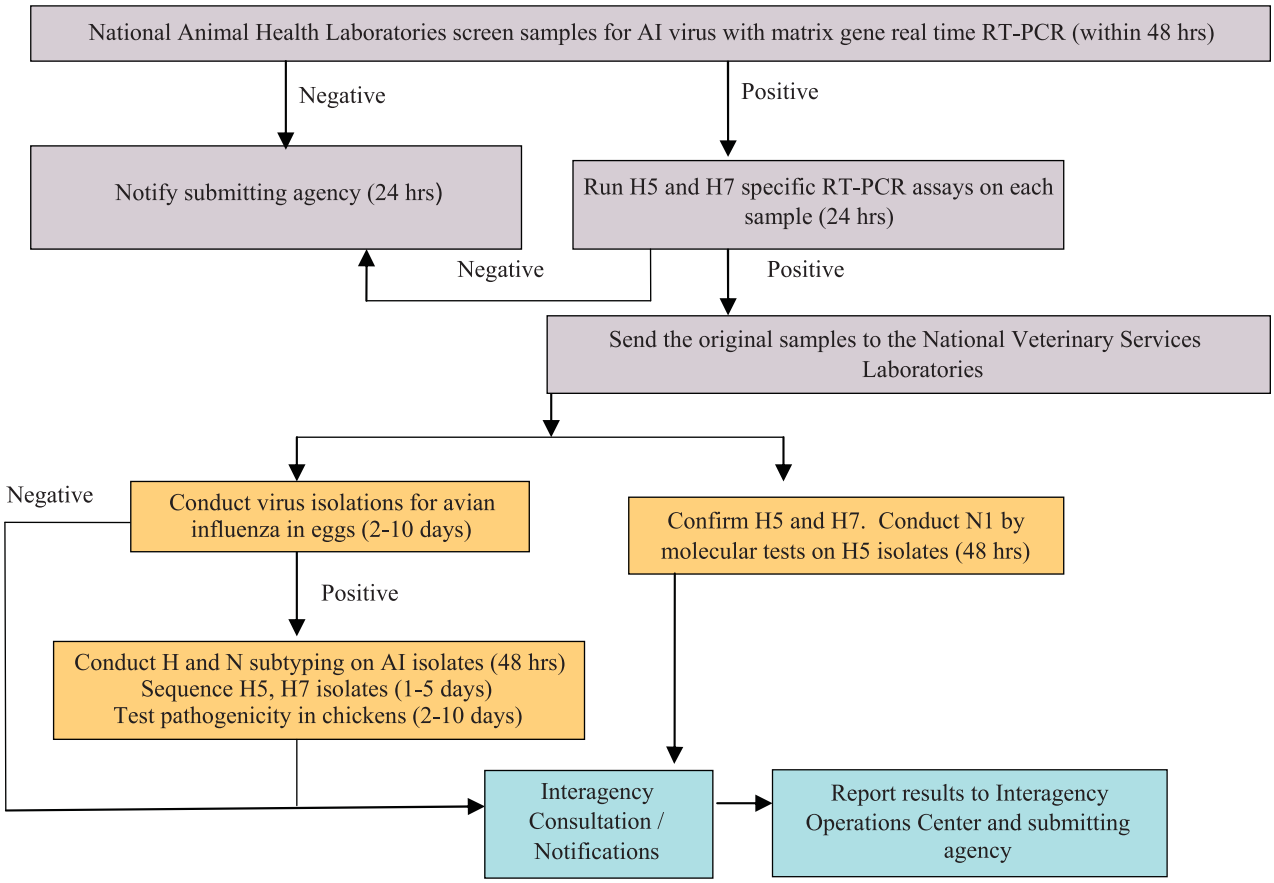


**Figure 1** Testing procedure for samples collected through the United States of America Early Detection System for Highly Pathogenic H5N1 Avian Influenza in Wild Migratory Birds.

method of surveillance compared to live bird sampling. Fecal sampling does not require the same level of skill to implement as live-bird sampling and can be implemented in rural and urban habitats. However, wild bird fecal samples must be fresh (i.e. within 24 h before desiccation and extended exposure to sunlight), might contain environmental contaminants that adversely impact diagnostic analyses, and can be difficult to obtain from some species of waterfowl that spend considerable amounts of time foraging and defecating in water. Exceptions are species such as Canada geese (*Branta canadensis* L., 1758) and snow geese (*Chen caerulescens* L., 1758) that spend significant time foraging and defecating on land. Additionally, although detection of AIVs in fecal samples is useful in determining the presence of viruses in the environment, the species infected might be difficult to determine if the collector does not observe the birds defecating. In the event of a HPAIV detection in feces, these limitations will require subsequent sampling of the wild bird populations in the area to allow for predictions of viral spread.

## Diagnostics

Swab samples were collected from birds and wild bird fecal samples. Bird samples were initially screened at 1 of 43 participating National Animal Health Laboratory Network facilities. This network is a partnership of state and federal laboratories across the USA that have been certified by the National Veterinary Services Laboratories (NVSL), the US OIE (World Organization for Animal Health) Reference Laboratory for AIV diagnostics. Swabs were initially tested by real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) using the matrix gene assay (Spackman *et al*. 2002). The matrix gene rRT-PCR assay was capable of detecting all 16 hemagglutinin and 9 neuraminidase subtypes. Matrix gene rRT-PCR-positive samples were further characterized by H5-specific and H7-

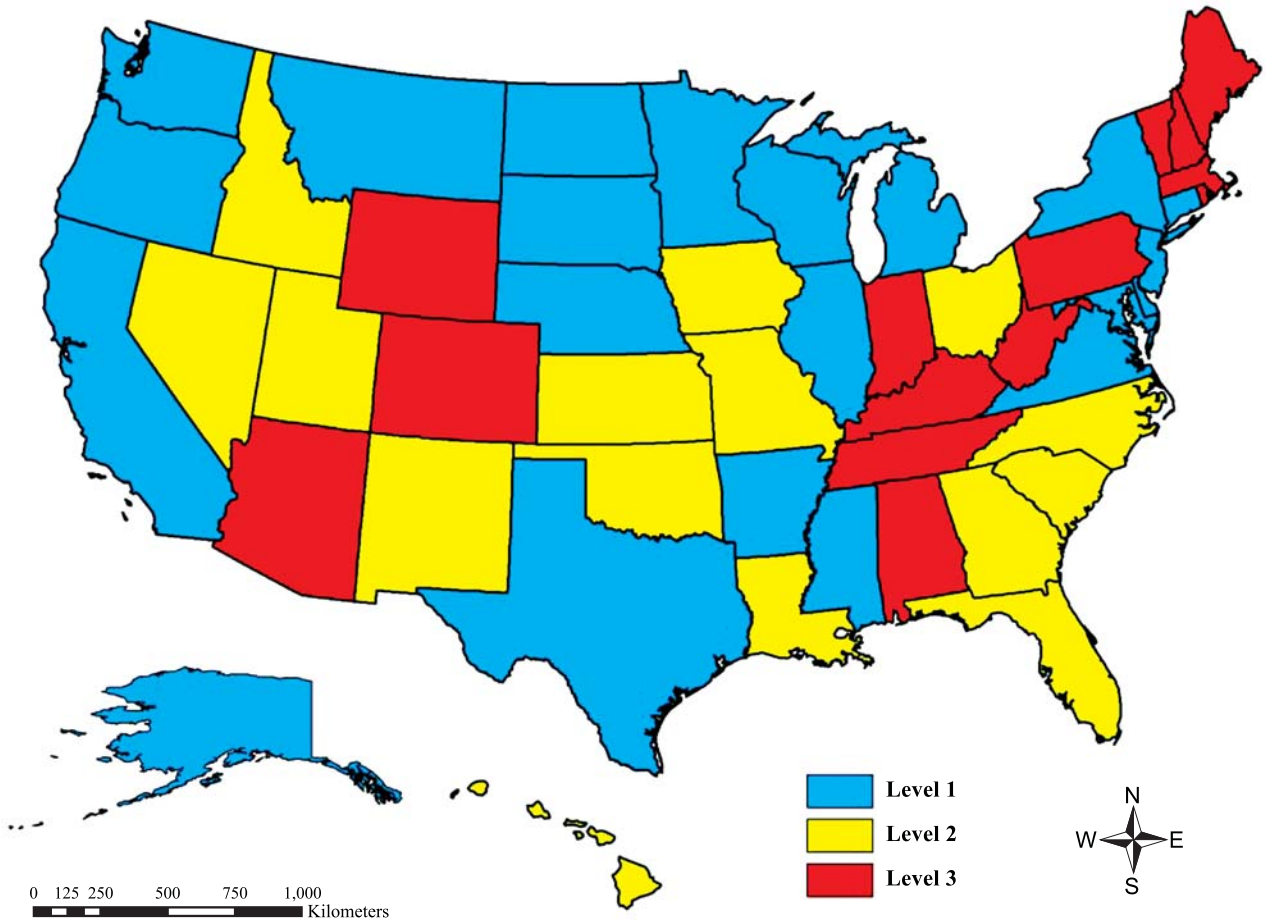


**Figure 2** United States Department of Agriculture priority ranking system for the detection of highly pathogenic H5N1 avian influenza in wild birds. Sampling goals were highest in Level 1 states followed by Level 2 and 3 states, respectively.

specific rRT-PCR assays (Spackman & Suarez 2008). Positive H5 or H7 rRT-PCR samples were express shipped to the NVSL within 24 h of a presumptive finding (Fig 1). Specific rRT-PCR assays, virus isolation, subtyping and pathogenicity tests were performed at the NVSL according to international guidelines (OIE 2008; Swayne *et al*. 2008).

Wild bird fecal samples were screened by rRT-PCR at the United States Department of Agriculture (USDA) Wildlife Services National Wildlife Research Center using a modified assay based on Spackman *et al*. (2003). Positive H5 and H7samples were forwarded to the NVSL for virus isolation, subtyping and pathogenicity testing, as described above. Additional subtyping was performed by amplifying hemagglutinin genes and sequencing analysis (Van Dalen *et al*. 2008).

## USDA IMPLEMENTATION OF THE USA STRATEGIC PLAN

The USDA and the Department of the Interior were the lead federal agencies responsible for working with tribal and state partners to implement the USA Strategic Plan. In coordination with these partners, the USDA Wildlife Services prioritized all 50 states according to known distributions of AIVs in wild birds, species-specific migratory pathways, geographic size and location of each state, wetland habitat and their juxtaposition with coastal shorelines, input from waterfowl biologists and flyway councils, and band recovery data (Fig 2). Target sample numbers were highest for priority Level 1 states, followed by Level 2 and 3 states, respectively.

Sampling was conducted during a biological year (BY)

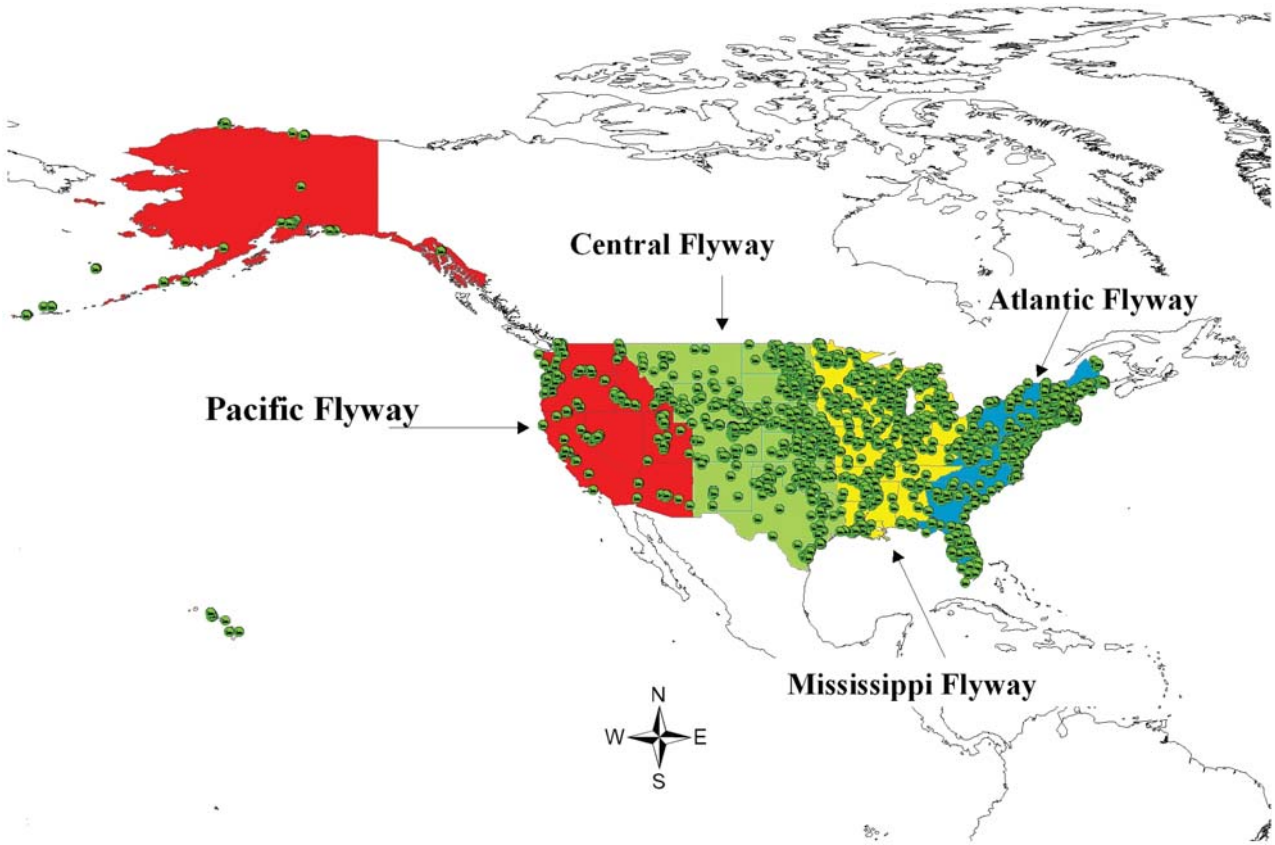


**Figure 3** Locations of wild birds sampled by the United States Department of Agriculture, and state and tribal wildlife agencies from 1 April 2006 to 31 March 2009.

beginning 1 April and ending 31 March. All reports of sick or dead birds (i.e. morbidity/mortality events) were investigated regardless of species. Separate tracheal and cloacal swabs were collected from each bird sampled in these events, and placed into separate tubes to preserve the greatest chance of isolating HPAIV and accurately describing the pathogenesis in affected birds.

During BY06 (i.e. 1 April 2006–31 March 2007), a cloacal sample was collected from each apparently healthy bird (i.e. hunter-killed, live-captured and sentinel birds) using a sterile dacron-tipped swab (Puritan, Puritan Medical Products Company LLC, Guilford, Maine, USA) and placed into a glass vial with 3 mL of brain–heart infusion media (Becton Dickinson, Sparks, Maryland, USA). Samples were stored in coolers with ice immediately after collection, transferred to refrigerators, and usually shipped within 24 h to one of the National Animal Health Laboratory Network facilities for rRT-PCR testing (Fig 1). Cloacal samples were tested in pools of up to five swabs collected from a single species, location and time.

During BY07 and BY08, separate cloacal and oropharyngeal swabs were collected from each apparently healthy bird (i.e. hunter-killed, live-captured and sentinel birds) sampled, and combined into one tube with 3 mL of brain–heart infusion media. Immediately after collection samples were transferred to refrigerators, and usually shipped within 24 h to screening laboratories. Pooling of samples was not conducted during BY07 and BY08 and combined cloacal and oropharyngeal samples from individual birds were tested separately.

In total, 50 000 wild bird fecal samples were collected in all 50 states during BY06. Based on a risk assessment using the BY06 data and an analysis of bird band recovery data, the sample size was reduced to 25 000 collected from 31 states in BY07 and BY08 (Doherty & Wilson 2009). Fecal samples were collected by inserting the swab into fecal material deposited on the ground (USDA 2008). Swabs were stored in individual tubes and then pooled in the laboratory for analysis (up to five swabs per location). Viral transport media in sample tubes was BA-1 with anti-

**Table 1** Number of real-time reverse transcriptase-polymerase chain reaction positive H5 avian influenza detections in wild bird species sampled by the United States Department of Agriculture, and state and tribal wildlife agencies from 1 April 2006 to 31 March 2009

| Species | 1 April 2006–31 March 2007 | 1 April 2007–31 March 2008 | 1 April 2008–31 March 2009 |
|---|---|---|---|
| *Aix sponsa* | 0 | 1 | 6 |
| *Anas acuta* | 20 | 17 | 43 |
| *Anas Americana* | 26 | 19 | 19 |
| *Anas clypeata* | 13 | 27 | 29 |
| *Anas crecca* | 28 | 37 | 68 |
| *Anas discors* | 4 | 6 | 24 |
| *Anas fulvigula* | 0 | 5 | 1 |
| *Anas platyrhynchos* (sentinel) | 7 | 0 | 0 |
| *Anas platyrhynchos* (wild) | 134 | 136 | 330 |
| *Anas rubripes* | 3 | 13 | 8 |
| *Anas strepera* | 2 | 9 | 9 |
| *Anser albifrons* | 0 | 0 | 1 |
| *Arenaria interpres* | 0 | 5 | 0 |
| *Aythya affinis* | 0 | 1 | 2 |
| *Aythya Americana* | 0 | 1 | 0 |
| *Aythya collaris* | 1 | 2 | 1 |
| *Aythya marila* | 0 | 1 | 0 |
| *Aythya valisineria* | 0 | 1 | 0 |
| *Branta canadensis* | 8 | 7 | 13 |
| *Branta hutchinsii* | 1 | 8 | 2 |
| *Bucephala albeola* | 0 | 3 | 5 |
| *Bucephala clangula* | 1 | 3 | 1 |
| *Cairina moschata* | 1 | 0 | 0 |
| *Chen caerulescens atlanticus* | 0 | 0 | 1 |
| *Chen caerulescens caerulescens* | 1 | 1 | 3 |
| *Chen rossi* | 0 | 0 | 1 |
| *Cygnus columbianus* | 1 | 1 | 0 |
| *Cygnus olor* | 2 | 1 | 0 |
| *Larus argentatus* | 0 | 1 | 0 |
| Other | 0 | 0 | 0 |
| *Oxyura jamaicensis* | 0 | 0 | 2 |
| Total | 253 | 306 | 569 |

biotics in BY06 and BY07 and brain–heart infusion media without antibiotics in BY08. Immediately after collection, samples were stored on ice packs and shipped to diagnostic laboratories at 4°C.

## EPIDEMIOLOGICAL ANALYSIS

To demonstrate freedom of HPAIV H5N1 in the USA wild, migratory bird population, a post hoc analysis on the number of wild bird and fecal samples collected during BY06-08 was conducted using FreeCalc v.2.0 (Cameron & Baldock 1998) to test the null hypothesis that HPAIV was present in the population at the minimum expected prevalence (≥0.001%). Freedom of disease was calculated using the infinite population probability formula with a = 0.01 and b = 0.01. Test sensitivity and specificity were set at 73.4 and 99.8%, respectively. Population size was set at 50 million and the infinite population threshold was set at 10 000 individuals.

Apparent prevalence of AIV in wild birds was calculated as the proportion of animals from the survey that tested matrix gene positive by rRT-PCR at a National Animal Health Laboratory Network facility or at the National Wildlife Research Center in the case of wild bird fecal samples. Apparent prevalence of H5 and H7 subtypes was calculated as the proportion of animals from the survey that tested positive by rRT-PCR at the NVSL.

## RESULTS

From 1 April 2006 through 31 March 2009, the USA collected 367 834 wild bird and wild bird fecal samples for AIV testing as part of the Interagency Wild Bird HPAI Early Detection System. The USDA, with its state and tribal cooperators, collected 314 654 (86%) of the samples in the USA, with wild bird and fecal samples accounting for 213 115 and 101 539 of the total, respectively. More than 250 species of wild birds in all 50 states, Guam, Puerto Rico and the Caribbean islands were sampled; however, 86% were collected from dabbling ducks, geese, swans and shorebirds. The remaining 14% were collected from a variety of other species. No wild bird or fecal sample tested positive for HPAIV.

Of the 213 115 wild bird samples collected by the USDA, 31, 27, 21 and 21% were collected from the Atlantic, Pacific, Central and Mississippi flyways, respectively (Fig 3). The majority of the samples (68%) were collected using the hunter-harvest collection strategy, followed by live wild bird (30%), morbidity and mortality events (2%), and sentinel species (<1%). The apparent prevalence of AIV in samples collected from wild birds during BY07 and BY08 was 9.7 and 11.0%, respectively. We were unable to estimate apparent prevalence in wild birds in BY06, because matrix gene rRT-PCR testing was only conducted on pooled samples in that year.

There were 1760 wild bird samples that screened positive for H5 or H7 AIV by rRT-PCR at a National Animal Health Laboratory Network facility. All states except Hawaii had at least 1 H5 positive sample during the 3-year surveillance effort (Fig 4). The NVSL confirmed 1128 H5 (Table 1) and 118 H7 (Table 2) positives by rRT-PCR from over 30 different species of wild migratory birds. All H7 positive samples were collected in BY08. Apparent prevalence of H5 and H7 AIV based on confirmed rRT-PCR results across all species was 0.5 and 0.06%, respectively. Virus was isolated from 426 (25%) of the wild bird samples that screened positive for H5 or H7. Of these, H5 subtypes were isolated from 13 species of wild birds, and H7 subtypes were isolated from 11 species. There were 9 different H5 subtype combinations and 8 different H7 subtype combinations identified by virus isolation. Hemagglutinin groups represented in these viruses were H1-H8, H10 and H11; all 9 neuraminidase groups were represented in the viruses isolated (Pedersen *et al*. 2009).

Of the 101 539 wild bird fecal samples collected, 27, 28, 21 and 23% were collected from the Atlantic, Pacific, Central and Mississippi flyways, respectively. There were 4.0, 6.7 and 4.7% matrix gene positive pools for BY06, BY07 and BY08, respectively. The NVSL confirmed 0.01, 0.16 and 0.02% positive H5 fecal pools by rRT-PCR in BY06, BY07 and BY08, respectively. No pools were confirmed H7 positive by NVSL using rRT-PCR.

The freedom from disease analysis indicated that the probability of observing an HPAIV positive reactor in a sample of 367 834 from a population of 50 million wild birds with a disease prevalence of 0.001% was $P = 0.000000$.

**Table 2** Number of real-time reverse transcriptase-polymerase chain reaction real-time reverse transcriptase-polymerase chain reaction positive H7 avian influenza detections in wild bird species sampled by the United States Department of Agriculture, and state and tribal wildlife agencies from 1 April 2008 to 31 March 2009

| Species | 1 April 2008–31 March 2009 |
|---|---|
| *Anas acuta* | 2 |
| *Anas americana* | 1 |
| *Anas clypeata* | 19 |
| *Anas crecca* | 34 |
| *Anas discors* | 11 |
| *Anas platyrhynchos* (domestic sentinel) | 3 |
| *Anas platyrhynchos* (wild) | 31 |
| *Anas strepera* | 1 |
| *Aythya collaris* | 1 |
| *Branta canadensis* | 2 |
| *Bucephala albeola* | 2 |
| *Cygnus olor* | 11 |
| Total | 118 |

## DISCUSSION

The USA Strategic Plan was successfully developed and implemented in response to the spread of HPAIV H5N1. This strategy capitalized on existing infrastructure and expertise at state and federal agriculture and natural resources agencies. The USA effort, combined with the Canadian and Mexican surveillance systems, represented the largest coordinated wildlife disease surveillance program ever implemented. During BY06–08, over 379 000 samples were collected from wild birds throughout North America and results were shared among all three countries. Coordination of each country's surveillance system was accomplished through the establishment of a trilateral HPAIV working group in 2006. This group met periodically to re-evaluate the continental surveillance of AIVs in wild birds, and ensured an appropriate sampling distribution in all four major flyways given available resources.

Results were adequate to reject the null hypothesis and conclude that the US population of wild birds was free of

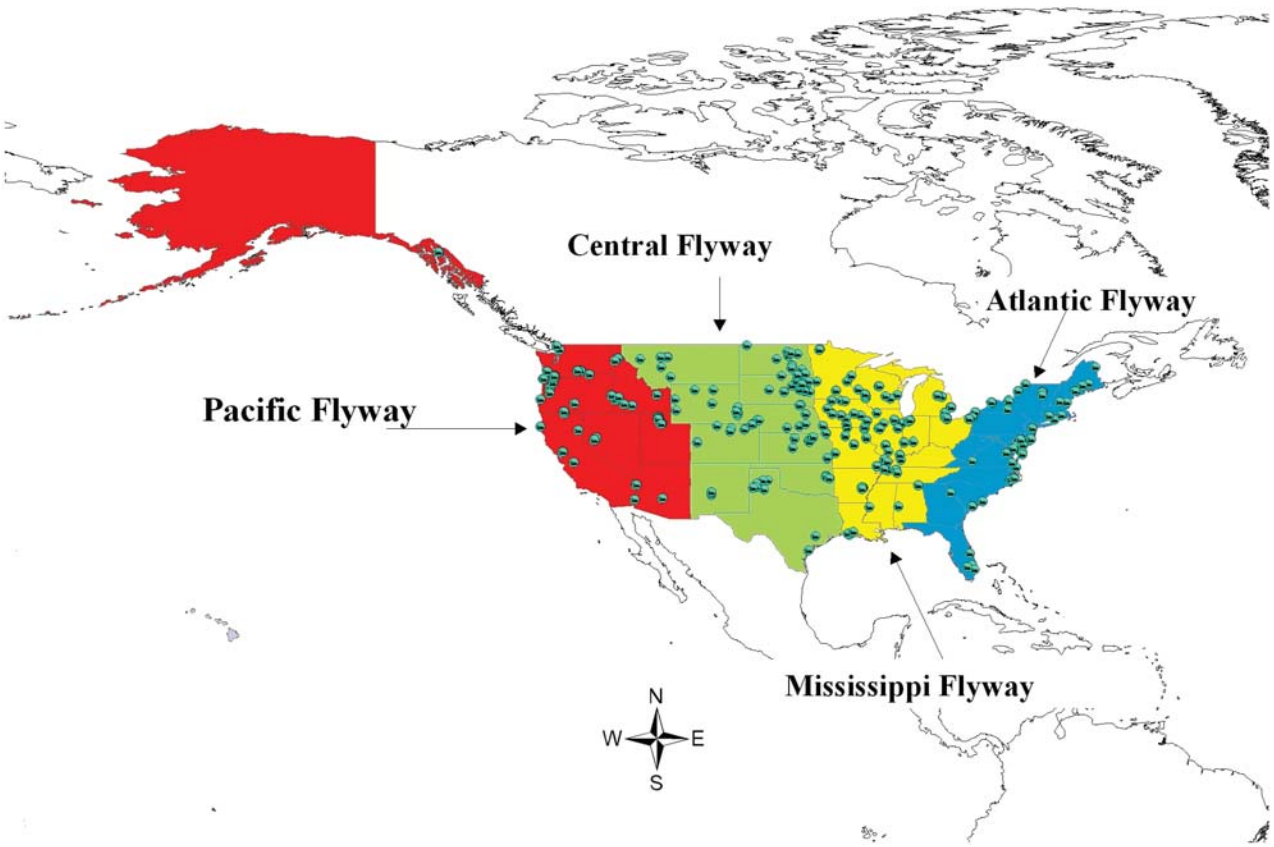


**Figure 4** Locations of H5 avian influenza positive samples collected by the United States Department of Agriculture, and state and tribal wildlife agencies from 1 April 2006 to 31 March 2009.

HPAIV (given the expected minimum prevalence of 0.001%) at the 99.9% confidence level during BY06-08. Although no HPAIV was detected in wild birds, the system demonstrated its capability of identifying H5 and H7 AIVs within 48 h of sampling. Additionally, preliminary sequencing of the hemagglutinin cleavage site by NVSL of all presumptive H5 and H7 positive samples within 36 h of initial test results demonstrated that the surveillance system was capable of rapidly detecting an introduction of HPAIV H5N1 by wild birds into the USA.

Early detection systems for potential introduction of HPAIV H5N1 by migratory birds into North America were supplemented with collaborative surveillance systems in eastern Russia, Greenland and Iceland. The USDA worked closely with the Russian Federal Centre for Animal Health and Ministry of Natural Resources to conduct sampling for AIV in snow geese on Wrangel Island Nature Reserve. Most snow geese that breed on Wrangel Island migrate through Alaska and Canada, and spend winter in the western USA (Ely *et al*. 1993; Armstrong *et al*. 1999). In Greenland, the USDA collaborated with the Technical University of Denmark, Aarhus University, the Danish Veterinary and Food Administration, and the Greenland Home Rule authorities to conduct AIV surveillance of wild birds in the western and southern portion of the country; since 2007, over 3000 birds have been tested. Finally, the Canadian Cooperative Wildlife Health Center conducted surveillance for AIVs in wild birds in Iceland (CCWHC 2007). These efforts, combined with the programs in Canada, Mexico and the USA, provided comprehensive surveillance of migratory birds in the North American flyways.

In addition to its capability of detecting HPAIV viruses, the system developed by the USDA and its cooperators provided valuable insights on AIV circulating in wild bird reservoirs throughout the USA (Pedersen *et al*. 2009). Although such results had been inferred from previous work, the numerous variables (e.g. temporal and latitudinal gradients, host immunocompetence and environmental persistence) influencing AIV infection made it difficult to determine which viruses were circulating within wild bird

populations at national and continental scales. This information is necessary for understanding and quantifying pathogen transmission within and among host species (Crowl *et al*. 2009). Large-scale surveillance projects such as the one undertaken in this effort will improve our understanding of the ecological parameters involved in the maintenance and transfer of AIVs from natural reservoirs to humans, which is an important component for developing methods to prevent future pandemics (Webster *et al*. 1992).

It is generally recognized that countries conducting comprehensive disease surveillance in wildlife populations are more likely to understand the epidemiology of specific infectious pathogens and zoonotic disease outbreaks. These countries are better equipped and prepared to develop solutions that will protect humans, agriculture and wildlife. Consequently, active surveillance for diseases of animal or public health concern in wildlife, such as HPAIV, is particularly beneficial to national and international interests. The OIE encourages all countries to develop and maintain wildlife disease surveillance systems that complement and support human health and agricultural animal disease programs.

Development and implementation of the USA Strategic plan has provided important ancillary benefits toward improved comprehensive wildlife disease surveillance. The number of wildlife biologists trained to investigate morbidity and mortality events, and to conduct active surveillance programs for diseases was increased nationwide. Diagnostic laboratories certified to conduct AIV testing as part of the National Animal Health Laboratory Network were increased, improving the capability of the USA to rapidly detect introductions of HPAIV as well as other exotic diseases. Enhanced communication protocols for reporting test results of diseases of concern in wildlife were developed and implemented. Critical field equipment necessary for conducting disease surveillance in wildlife and responding to disease outbreaks was purchased. A national wild bird tissue archive was created by the USDA to provide a resource for future studies on AIV and other diseases. Finally, the benefits of improved coordination among wildlife biologists and veterinarians, agricultural veterinarians and laboratory diagnosticians resulting from the HPAIV wild bird surveillance effort cannot be underestimated. These enhancements to the wildlife disease surveillance efforts in the USA will continue to safeguard the health of wild and domestic animals, as well as the public at large.

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